

***Amendments to the Specification***

Please replace paragraph [0018] on page 5 with the following amended paragraph.

Figure 1 shows rat ferritin heavy chain exon sequences (SEQ ID NOs: 1, 4 and 7) and amino acid sequences (SEQ ID NOs: 2, 5, 8 and 9). The complementary strand is also shown (SEQ ID NOs: 3, 6 and 10).

Please replace paragraph [0020] on page 5 with the following amended paragraph.

Figure 3 illustrates the deletion of exons 2, 3, and 4 from pFerX1 and insertion of a polylinker (SEQ ID NOs: 40-41) to generate plasmid pFerX2.

Please replace paragraph [0022] on page 5 with the following amended paragraph.

Figure 5 A-B illustrates the removal of exons 2 through 4 of the ferritin heavy chain gene from cosmid 15A using PCR fusion (SEQ ID NOs: 11, 15, 16 and 17) and amino acid sequences (SEQ ID NOs: 12-14).

Please replace paragraph [0029] on page 5 with the following amended paragraph.

Figure 12 illustrates the sequence of the transcribed region of the pFerX8 and pFerX9 plasmids (SEQ ID NO: 18).

Please replace paragraph [0078] on pages 19-20 with the following amended paragraph.

The exon 1 coding region was deleted from pFerX2, leaving the ATG initiation codon and the following splice donor intact to generate plasmid pFerX3. Figure 4 illustrates that the deletion of the exon 1 coding region was accomplished by isolating the BamHI-BspHI (2515-2719) and NcoI-BamHI (2830-2515) fragments from pFerX2. BspHI and NcoI generate compatible overhangs which permitted the resulting fragments to be ligated together to generate pFerX3. As a result of this manipulation, exon 1 of the vector was changed from:

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      BspHI
CCAGCCGCCATC ATG ACC ACC GCG TCT CCC TCG CAA GTG CGC CAG AAC TAC CAC CAG GAC TCG GAG GCT
GGTCGGCGGTAG TAC TGG TGG CGC AGA GGG AGC GTT CAC GCG GTC TTG ATG GTG GTC CTG AGC CTC CGA
      ▶Met Thr Thr Ala Ser Pro Ser Gln Val Arg Gln Asn Tyr His Gln Asp Ser Glu Ala

                                     NcoI      Splice Donor
GCC ATC AAC CGC CAG ATC AAC CTG GAG TTG TAT GCC TCC TAC GTC TAT CTG TCC ATG GTGAGTGCAGCCT
CGG TAG TTG GCG GTC TAG TTG GAC CTC AAC ATA CGG AGG ATG CAG ATA GAC AGG TAC CACTCACGCCGGA
▶Ala Ile Asn Arg Gln Ile Asn Leu Glu Leu Tyr Ala Ser Tyr Val Tyr Leu Ser Met
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(SEQ ID NO: 19) and amino acid sequence (SEQ ID NO: 20). The complementary strand is also shown (SEQ ID NO: 21)

to:

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                                     Splice Donor
CCAGCCGCCATC ATG GTGAGTGCAGCCT      SEQ ID NO: 22
GGTCGGCGGTAG TAC CACTCACGCCGGA      SEQ ID NO: 23
      ▶Me t
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Please replace paragraph [0079] on page 20 with the following amended paragraph.

Deletion of the exon 1 IRE was accomplished by replacing the SacII-EagI (2575-2639) fragment in pFerX3 with a linker that does not contain the IRE (but creates a 5' KpnI site for screening) to generate plasmid pFerX4. As a result of this manipulation, exon 1 of the vector was changed from (IRE underlined):

SacII (2575)	EagI (2639)
CAGAGTCGCCGCGGTTT <b>CCTGCTTCAACAGTGCTTGAACGGA</b> ACCCGGTGCTCGACCCCTCCGACCCCGTCCGGCCGCTTTGAGCC	
GTCTCAGCGCGCCAA <b>AGGACGAAGTTGTCACGA</b> ACTTGCTTGGGCCACGAGCTGGGGAGGCTGGGGGCAGGCCGGCGAAACTCGG	

SEQ ID NOs: 24-25. The complementary strand is shown (SEQ ID NO: 25)

to (linker shown in bold):

KpnI (2579)	EagI (2611)
SacII (2575)	
CAGAGTCGCCGCGGT <b>ACCGGTGCTCGACCCCTCCGACCCCGTCCGGCCGCTTTGAGCC</b>	
GTCTCAGCGCGCC <b>ATGGCCACGAGCTGGGGAGGCTGGGGGCAGGCCGGCGAAACTCGG</b>	

SEQ ID NOs: 26-27 respectively. The complementary strand is shown (SEQ ID NO: 27)

Please replace paragraph [0080] on pages 20-21 with the following amended paragraph.

A PCR fusion product was generated in a three step procedure to replace exons 2 though 4 with a polylinker containing SwaI and NotI, while maintaining the proximal 5' regulatory sequences and proximal 3' regulatory sequences of the ferritin heavy chain gene. As shown in Figure 5(A), the first PCR used cosmid 15A (Figure 2) as a template. Primer locations for primers Fer1 and Fer4 are indicated by arrows. The "priming" region for primers FN1 and FN2 are also indicated by bars. In the second step, shown in Figure 5(B), a Fer1-FN2 PCR product was generated.. The location of the "priming" region of primer Swa-2 is indicated. In the third step, shown in Figure 5(C), a FN1-Fer4 PCR product was generated. The location of the "priming" region of primer Swa-1 is

indicated. In the fourth and final step, as shown in Figure 5(D), the final PCR fusion product was generated by using the Fer1-Swa-2 and Swa-1-Fer4 products as templates and the Fer1 and Fer4 primers. The HpaI–AatII fragment was isolated from this product for insertion into the HpaI and AatII sites of pFerX4 to generate plasmid pFerX5 (see Figure 6). The PCR fusion reactions used in the first three steps to generate the SwaI–NotI polylinker are shown in TABLE 1.

TABLE 1

	Template(s)	5' primer	3' primer
First PCR	Cosmid 15A	Fer1	FN2
	Cosmid 15A	FN1	Fer4
Second PCR	Fer1/FN2 product	Fer1	Swa-2
	FN1/Fer4 product	Swa-1	Fer4
Third PCR	Fer1/Swa-2 & Swa-1/Fer4 products	Fer1 or Fer3	Fer4

The PCR primers are shown below, where the polylinker sequence is shown in bold, and the complementary sequences between FN1 and FN2 or between Swa-1 and Swa-2 are shown underlined.

FN1                      NheI   NotI                      AatII  
ACTTTCAGCTGCTAGCGGCCGCGCTGACGTCCCCAAGGCCAT      SEQ ID NO: 28

FN2                      NotI           NheI  
ACGTCAGCGCGGCCGCTAGCAGCTGAAAGTGGAAAGGGTAT      SEQ ID NO: 29

Swa-1                      SwaI                      NotI                      AatII  
CTTTCATTAAATCTGCTAGCGGCCGCTGACGTC      SEQ ID NO: 30

Swal  
Swa-2     T A G C A G A T T T A A A T G G A A G G G T A T T T G T T A T T G A T C

SEQ ID NO: 31

Please replace paragraph [0083] on page 22 with the following amended paragraph.

The polylinker

BglII     BstBI  
CTGTGAGATCTGTTCTGAATGG  
TGCAGACACTCTAGACAAGCTTACCAGCT  
AatII     Sall  
compatible     compatible

SEQ ID NO: 32

SEQ ID NO: 33

was inserted into the Sall-AatII sites of pFerX5.1 to generate plasmid pFerX6. The polylinker includes both BglII and BstBI sites and was designed to receive the distal 3' flanking sequences of the ferritin heavy chain gene.

Please replace paragraph [0090] on page 23 with the following amended paragraph.

The sequence of the vector polylinker and the original sequence at the 5' end of exon 2 that needs to be recreated to regenerate the splice donor are shown in Figure 5. Thus, the 5' primer should include a CAG at the 5' end to recreate the natural splice donor followed by the coding region starting with the second amino acid (the ATG is already included in exon 1). The 5' end of the PCR product should be left blunt-ended for ligation with the Swal site. For example:

General 5' primer:

CAG NNN NNN NNN NNN NNN NNN NNN  
AA2 AA3 AA4 AA5 AA6 AA7 AA8

SEQ ID NO: 34

Primer for SEAP example:

CAG CTG CTG CTG CTG CTG CTG CTG CTG GGC

SEQ ID NO: 35

Please replace paragraph [0091] on pages 23-24 with the following amended paragraph.

The 3' primer should include a NotI site followed by the 3' end of the gene including the termination codon (opposite strand). The PCR product should be digested with NotI to generate an end compatible with the NotI site in the polylinker. For example:

General 5' primer:

NNNN GCGGCCGC NNN NNN NNN NNN NNN NNN NNN  
NotI 3' end of gene  
site

SEQ ID NO: 36

Primer for SEAP example (termination codon in bold):

TTTT GCGGCCGC AGC **TCA** TGT CTG CTC GAA GCG GCC

SEQ ID NO: 37

Please replace paragraph [0092] on page 24 with the following amended paragraph.

Ligation of the PCR product with the vector (digested with SmaI and NotI) does not recreate a SmaI site at the 5' end of the insert. Instead the ligated product contains a suitable splice acceptor at the "SmaI end." The inserted region will also contain the coding sequence from the second amino acid to the termination codon followed by the NotI site at the 3' end. For example:

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Reply to Office Action of April 2, 2008

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After ligation generally:

CCATTT CAG NNN NNN NNN // NNN NNN NNN GCGGCCGC TGACGT

SEQ ID NO: 38

Example for SEAP:

CCATTT CAG CTG CTG CTG // CAG ACA TGA GCGGCCGC TGACGT

SEQ ID NO: 39